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(54) PROCESS FOR PRODUCING HYDROXYETHYL
 STARCHES

(71) We, MORISHITA PHARMACEUTICAL CO., LTD., a Japanese body corporate, No. 29, 4-chome, Dosho-machi, Higashi-ku, Osaka-shi, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a hydroxyethyl starch having a molar ratio between the resulting hydroxyl group at the 2-position and those at the 6-position of 0.5 to 2.0, and to a process for the production thereof.

Conventional processes for producing a hydroxyethyl starch substituted with hydroxyethyl groups on carbon atom 2 of the glucose ring comprise reacting a hydroxyethylating agent such as ethylene oxide or ethylene chlorohydrin with starch or hydrolyzed starch in the presence of a catalytic amount of caustic alkali.

It is an object of the present invention to provide a hydroxyethyl starch having more substituted hydroxymethyl groups on carbon atom 6 than on carbon atom 2 of the glucose ring. Such hydroxyethyl starches have been found to be more effective as a plasma expander than hydroxyethyl starches having more hydroxyethyl groups on carbon atom 2 than on carbon atom 6 of the glucose ring and obtained by the above-described known process.

In recent years, blood substitutes, especially plasma expanders, have found extensive use due to the increase in the number of surgical operations and the frequent occurrence of traffic accidents. However, presently used plasma expanders Dextran—40 and Dextran—70 have a low antigenic activity which frequently causes fever and objectionable allergic reactions such as urticaria and tends to produce bleeding when the expander is transferred in a large amount. On the other hand, hydroxyethyl starches synthesized in 1934 by W. Ziese (*Z. Physiol. Chem.*, 299, 213 (1934)) are reported to be applicable as a plasma expander in 1957 by Widersheim *et al.* (*Arch. Int. Pharmacodyn.*, 111, 353 (1957)).

Hydroxyethyl starches have been prepared by the reaction of ethylene oxide with soluble starch in the presence of a catalytic amount of alkali. Although it had been difficult to locate accurately where the hydroxyethyl groups are substituted in the glucose ring of the thus-prepared starch, recent researches have revealed that the majority of the hydroxyethyl groups are substituted in the secondary alcohol on the carbon atom 2 of the glucose ring.

More specifically, the resulting ratio of 2-hydroxyethylglucose (hereinafter referred to as "2—O—HEG") to 6-hydroxyethylglucose (hereinafter referred to as "6—O—HEG"), namely the ratio of 2—O—HEG/6—O—HEG, has been found to be 5.20 by Belder *et al.* (*Carbohyd. Res.*, 10, 391 (1969)), 5.50 by Sivastava *et al.* (*Stärke*, 21, 181 (1969)), and 5.54 by Bollenbach *et al.* (*Cereal Chem.*, 46, 304 (1969)). From these ratios it is clear that a greater number of hydroxyethyl groups are substituted on carbon atom 2 than on carbon atom 6 of the glucose ring.

On the other hand, according to researches on the enzymatic activity of amylase on methyl ether starches, compounds having more substituents on the carbon atom 6 of the glucose ring are reported to be relatively more amenable to an enzymatic reaction than those having more substituents on the carbon atom 2 of the glucose ring (Y. Matsushima *et al.*, *J. Biochem.*, 64, 73 (1968)).

To produce hydroxyethyl starches having a molar ratio between the resulting hydroxyethyl groups at the 2-position and those at the 6-position (2—O—HEG/6—O—HEG) of 0.5 to 2.0, at least 2 moles of alkali is used per mole of starch or hydrolysed starch.

- 5 The molar ratio of alkali to starch during the reaction is calculated according to the following equation, and the ratio of the components, i.e. 2—O—HEG/6—O—HEG ratio of the hydroxyethyl starch obtained at the molar ratio of the alkali to starch thus calculated is given in Table 1. The table indicates that as already described, use of alkali as a reagent but not as catalyst as in the known process results in synthesis of hydroxyethyl starches having more substituents on the carbon atom 6 than in the carbon atom 2 of the glucose ring and that the molar ratio of alkali to starch must be at least 2.0 to achieve the foregoing ratio of 0.5 to 2.0 which is especially suitable for use as a plasma expander.

$$\text{molar ratio} = \frac{162}{\text{Amount of starch (g)}} : \frac{\text{Amount of sodium hydroxide (g)}}{40}$$

- 15 wherein 162 is the molecular weight of an hydroglucose unit and 40 is that of sodium hydroxide.

TABLE I

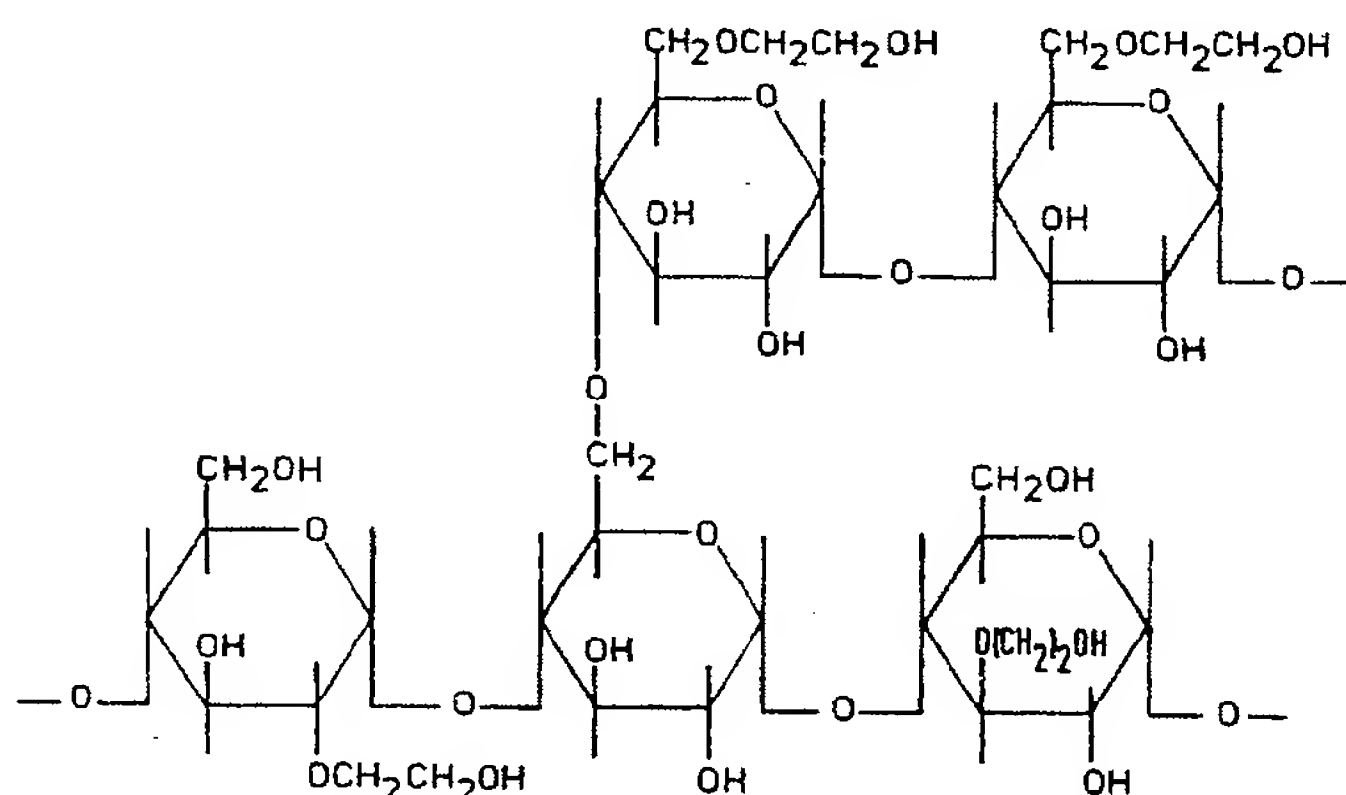
Molar ratio of alkali/starch	Molar ratio of 2—O—HEG/6—O—HEG	Degree of substitution with hydroxyethyl groups
0.1	10.8	0.68
0.2	9.8	1.06
0.5	5.9	1.13
1.0	4.0	0.94
2.0	2.0	0.87
5.0	0.80	0.58
7.0	0.5	0.43

Use of potassium hydroxide in place of sodium hydroxide gave similar results.

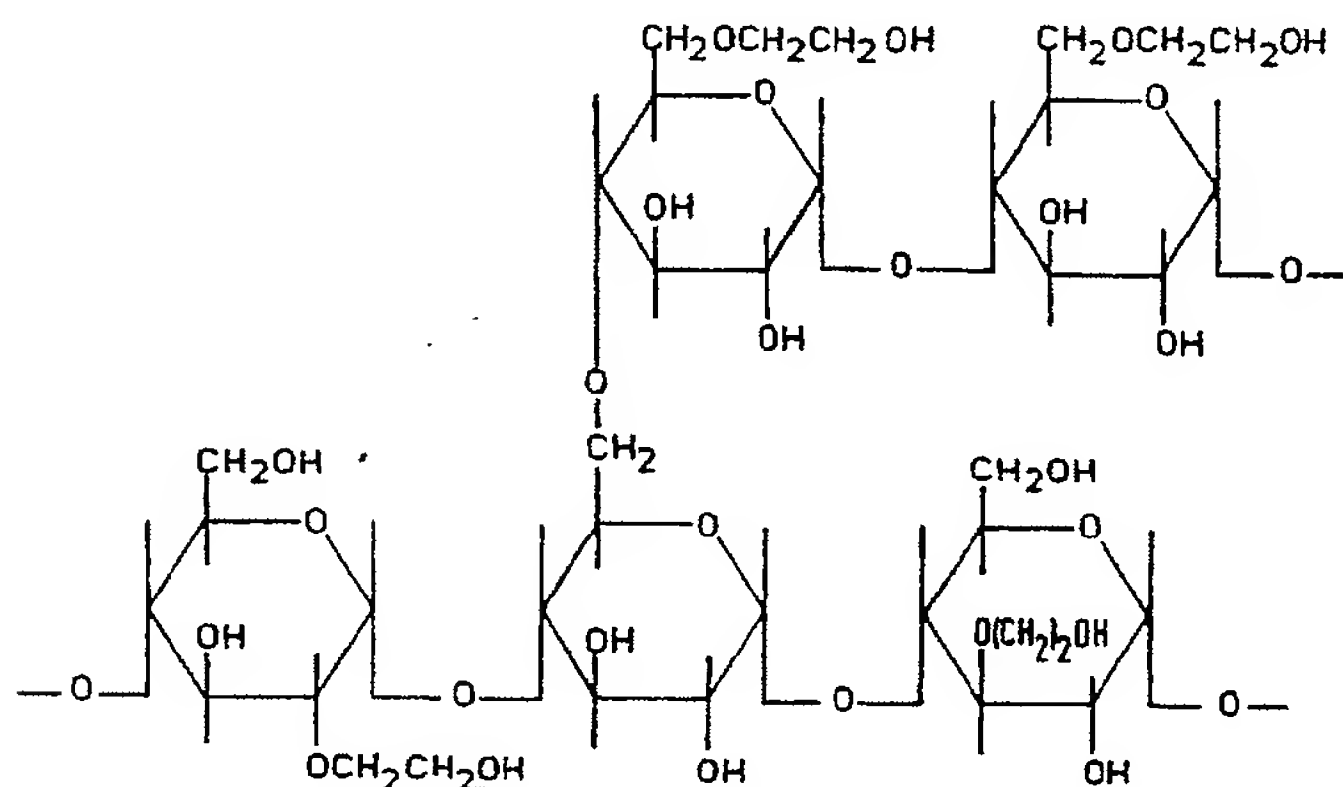
- 20 Table I shows the resulting molar ratios of 2—O—HEG/6—O—HEG and degrees of substitution (hereinafter referred to simply as "DS") achieved by using constant amount of starch and ethylene oxide and varying the amount of alkali used. DS is the fraction of available sites on the glucose residues actually substituted and is calculated according to the method of Morgan *et al.* (Ind. Eng. Chem. Anal. Ed., 18, 500 (1946)).

- 25 For reference, constant amounts of starch and alkali were used to observe the changes in the molar ratio of 2—O—HEG/6—O—HEG due to the differences in the amount of ethylene oxide introduced (i.e. differences in the DS value), but no changes were found.

- 30 According to the present invention there is provided a hydroxyethyl starch having a molar ratio between the resulting hydroxyethyl groups at the 2-position and those at the 6-position of 0.5 to 2.0, said starch being as shown in the partial formula:



Further according to the present invention there is provided a process for producing a hydroxyethyl starch having a molar ratio between the resulting hydroxyethyl groups at the 2-position and those at the 6-position of 0.5 to 2.0, said starch being as shown in the partial formula:



comprising the step of reacting a hydroxyethylating agent with starch or hydrolyzed starch in the presence of alkali, the molar ratio of alkali to starch being at least 2.0.

Examples of the starch or hydrolyzed starch which may be used as a starting material according to the process of this invention are corn starch, glutinous rice starch, wheat starch, potato starch, waxy maize starch, soluble starch and thin boiling starch. Particularly preferable are starches having a high amylopectin content. The alkalis to be used according to the present process are preferably sodium hydroxide and potassium hydroxide while alcoholates such as methylate and ethylate are also employable. Examples of hydroxyethylating agent are ethylene oxide and ethylene chlorohydrin.

Further irrespective of the concentration of alkali used, a catalytic amount of organic base may be added to the reaction system to lower the molar ratio of 2—O—HEG/6—O—HEG by 20 to 40% as compared with the case where the organic base is not used. Presumably, this is attributable to hydrogen bonding of the organic base with the hydrogen atom of the active hydroxyl groups on the carbon atom 2 of the starches, which impedes the substitution of hydroxyethyl ion at the 2-position.

By catalytic amount of organic base as referred to above is generally meant an amount not more than 0.5 mole per mole of starch or hydrolyzed starch (the molecular weight of anhydro-glucose unit thereof being 162) used as a starting material. Examples of organic bases usable in the present process are guanidine, pyridine, cyclohexylamine, ethylenediamine, triethanolamine, monoethanolamine, aniline, phenylenediamine, morpholine, piperidine, pyrrole, piperazine, phenylhydrazine, urea, methylamine, ethylamine, trimethylamine, triethylamine, semicarbazide, pyrrolidine, aminoguanidine and

aminophenol. Usable organic bases are not limited to these examples but any organic bases which are effective may be employed insofar as they do not produce an adverse effect such as hydrolysis of the resulting hydroxyethyl starches by acting thereon. Table II below shows specific results achieved with or without use of organic base as the catalyst.

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TABLE II

Sodium hydroxide amount used g.	Pyridine amount used g.	DS	Molar ratio of 2-O-HEG/6-O-HEG
0.2	—	0.89	8.52
0.2	0.2	0.72	6.21
0.5	—	1.13	5.90
0.5	0.02	0.97	4.62
0.5	0.1	0.83	4.16
0.5	0.2	0.78	3.89
0.5	0.6	0.24	4.27
0.5	2.0	0.089	4.36
1.0	—	0.94	4.00
1.0	0.2	0.81	3.21

Table II indicates that the use of an organic base in excess of a catalytic amount exerts an adverse effect on the molar ratio of the resulting components as well as on the DS value. This is presumably due to the reaction of hydroxyethylating agent with the excess amount of organic base. Accordingly, the organic base is used in a catalytic amount as previously described, generally in an amount not exceeding 0.5 mole per mole of starch used as a starting material. Preferable results will be achieved when it is used in a ratio of about 0.1 mole.

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An inorganic salt may be used in place of the organic base to synthesize hydroxyethyl starches having a much greater number of substituents at the 6-position than when alkali alone is used.

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Examples of the inorganic salt which may be used are sodium nitrite, sodium hydrogen phosphite, sodium phosphite, aluminium chloride, lithium chloride, titanate chloride, ferrous chloride, manganous chloride, potassium cyanate, potassium chlorate, sodium chloride, sodium chromate, sodium perchlorate, sodium hypophosphite, manganous bromide, sodium nitrate, sodium hypophosphate, sodium carbonate, sodium bicarbonate, sodium pyrosulfite, sodium pyrosulfate, boric acid, metaboric acid, aluminium iodide, sodium borate, aluminium sulfate, potassium aluminium sulfate, sodium thiosulphate, sodium sulphate, magnesium sulphate, potassium phosphate, disodium hydrogenphosphate and sodium phosphate. The inorganic salts are not limited to these examples but any inorganic salts can likewise be used, provided that they are effective and do not exercise an adverse effect on the starting material and on the hydroxyethyl starches. Preferable among these examples are lithium chloride, aluminium chloride, sodium sulfate, sodium thiosulfate, magnesium sulfate, sodium chloride and disodium hydrogenphosphate.

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Specific results achieved with or without using inorganic salts are given in Table III.

TABLE III

Starting material (soluble starch) g.	Sodium hydroxide (NaOH) g.	Sodium sulfate (Na ₂ SO ₄ ·10H ₂ O) g.	DS	Molar ratio of 2—O—HEG/ 6—O—HEG
4.05	2.0	—	0.87	2.04
4.05	2.0	8	0.80	1.53
4.05	2.0	16	0.93	1.23
4.05	2.0	32	0.78	0.85

Although the reaction according to the process of this invention is usually carried out in a container open to the atmosphere, it is possible to seal the container particularly when it is desired to improve the DS value of hydroxyethyl groups.

It will thus be apparent from the foregoing description that the process of the present invention is capable of producing hydroxyethyl starches having a greater number of substituents on the carbon atom 6 than on the carbon atom 2 of the glucose ring easily and relatively inexpensively. Moreover, the hydroxyethyl starches obtained according to the invention are particularly useful as plasma expanders.

The process for producing hydroxyethyl starches according to this invention are given in the following Examples.

Example 1.

4.05 g. of waxy maize starch is suspended in 80 c.c. of water containing 5.0 g. of sodium hydroxide. 3.2 g. of ethylene oxide is introduced into the resulting suspension in a gaseous state at 40°C. over a period of 3 hours. After the ethylene oxide has been introduced, the reaction is further continued with stirring for 2 hours at the same temperature. After cooling, 80 ml. of cation exchange resins are added to the reaction mixture, followed by stirring. The resins are then filtered off, and acetone is added to the filtrate obtained to precipitate the desired hydroxyethyl starch. The resulting precipitate is collected by filtration, and the precipitate is washed with acetone-ethanol to give 4.0 g. of hydroxyethyl starch (DS=0.58) as colorless crystals having a 2—O—HEG/6—O—HEG ratio of 0.78.

Example 2.

10 g of waxy maize starch is partially hydrolyzed with 12 ml. of 0.5 to 1.0N hydrochloric acid for 5 to 7 hours to prepare 9.8 g. of a thin boiling starch, 4.05 g. of which is then dissolved in 30 ml. of water containing 2.8 g. KOH. 3.2 g. of ethylene oxide is introduced into the solution in a gaseous state at a reaction temperature of 30°C under atmospheric pressure over a period of 3 hours. After cooling, cation exchange resins are added to the reaction mixture to remove alkali and the resin is filtered off. The filtrate is concentrated, followed by addition of acetone to give precipitation. The resulting precipitate obtained is washed with acetone ethanol to give 4.1 g. of hydroxyethyl starch (DS=0.75) as colorless powder having a 2—O—HEG/6—O—HEG ratio of 1.6.

Example 3.

2.0 g. of sodium hydroxide and 16 g. of sodium sulfate are dissolved in 30 ml. of water. 4.05 g. of soluble starch is added to the solution thus prepared, and 3.7 ml. of ethylene oxide is introduced in the gaseous state into the mixture at 40°C. with stirring for about 4 hours, followed by further stirring for 1 hour at the same temperature. After reaction, the reaction mixture is made acid with cation exchange resins (not higher than pH 1), to which anion exchange resins are further added for neutralization. After removing both resins from the neutralized reaction mixture, the neutralized mixture is concentrated at 50°C. under a reduced pressure. About 60 ml. of isopropyl alcohol or acetone is added to the concentrated mixture to precipitate the

desired product (hydroxyethyl starch). The precipitate is removed by filtration and thoroughly washed with acetone-ethanol to obtain 4.0 g. of hydroxyethyl starch (DS=0.93) as colorless powder having a 2—O—HEG/6—O—HEG molar ratio of 1.23 (see Table III).

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Example 4.

Reactions are conducted under the same conditions as in Example 3, using inorganic salts other than sodium sulfate. The results are given in Table IV. The products are obtained as colorless crystals in yields of about 90%.

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TABLE IV

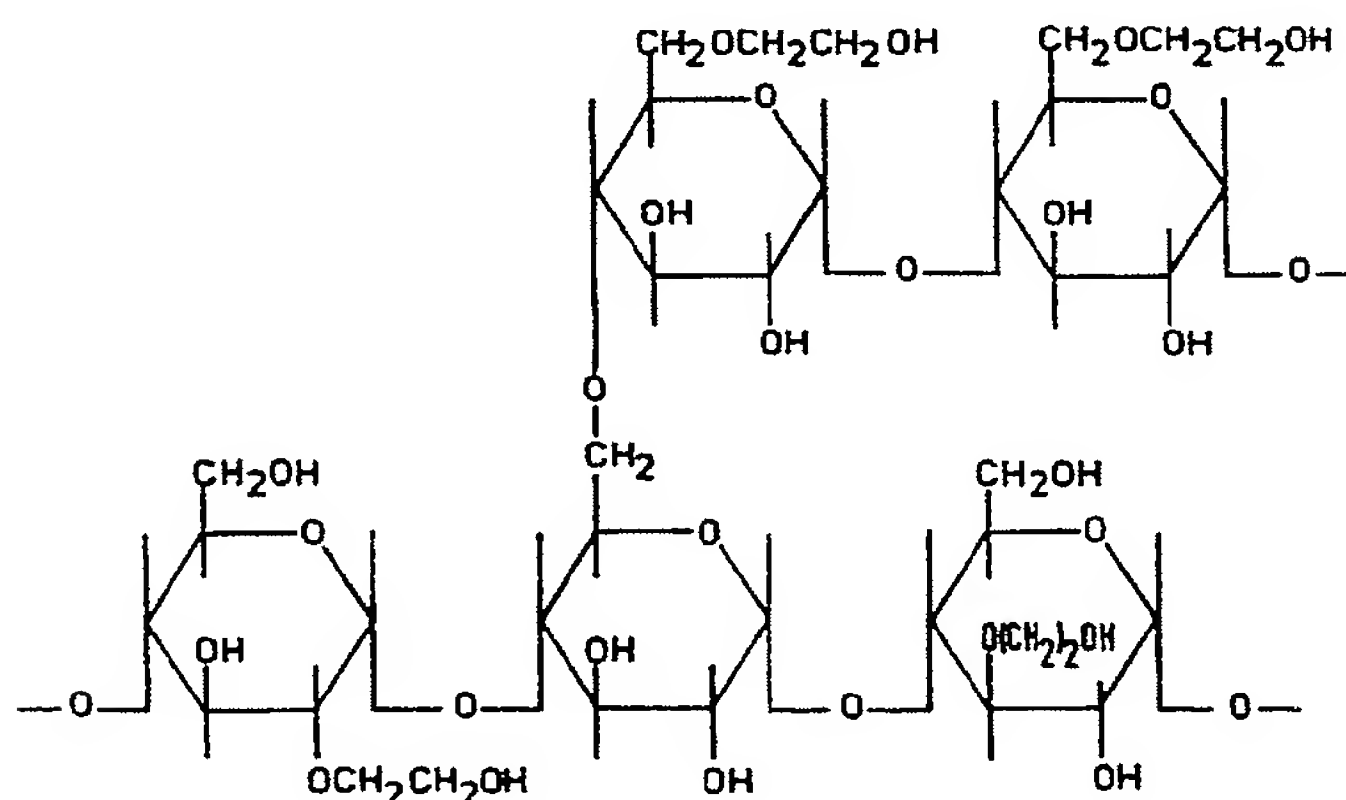
Sodium hydroxide (amount) g.	Organic base used	(amount) g.	DS	Molar ratio of 2—O—HEG/6—O—HEG
2.0	Lithium chloride	2.5	0.67	0.92
2.0	Aluminium chloride hexahydrate	12.0	0.14	1.04
2.0	Sodium thiosulfate pentahydrate	12.0	0.23	1.15
2.0	Potassium cyanate	5.0	0.76	1.03
2.0	Potassium sodium phosphate heptahydrate	14.0	0.54	1.18
2.0	Disodium hydrogenphosphate heptahydrate	13.4	0.65	1.21
2.0	Sodium carbonate	4.0	0.74	1.42

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WHAT WE CLAIM IS:—

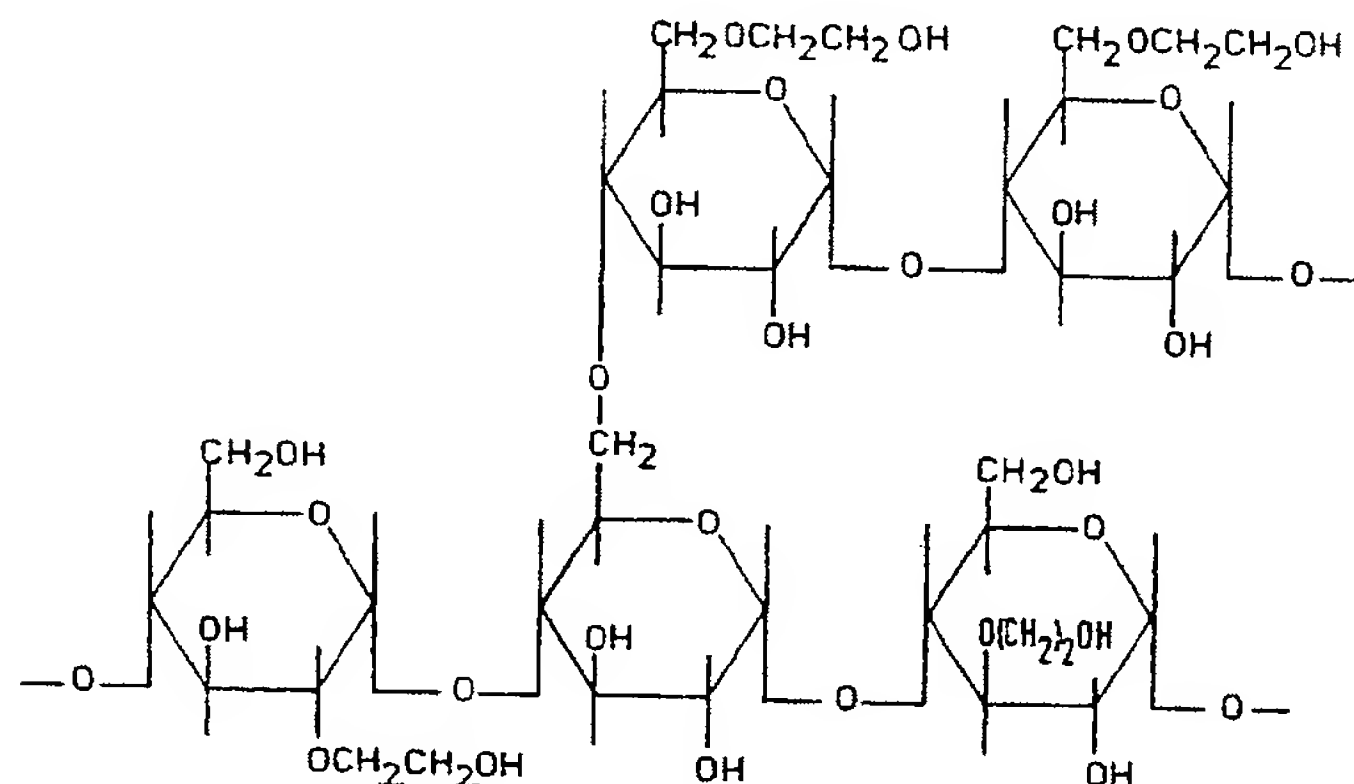
1. A hydroxyethyl starch having a molar ratio between the resulting hydroxyethyl groups at the 2-position and those at the 6-position of 0.5 to 2.0, said starch being as shown in the partial formula:

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2. A hydroxyethyl starch as claimed in claim 1, substantially as hereinbefore described.

3. A process for producing a hydroxyethyl starch having a molar ratio between the resulting hydroxyethyl groups at the 2-position and those at the 6-position of 0.5 to 2.0, said starch being as shown in the partial formula:



comprising the step of reacting a hydroxyethylating agent with starch or hydrolyzed starch in the presence of alkali, the molar ratio of alkali to starch being at least 2.0, said molar ratio being determined in accordance with the equation provided herein.

4. A process as claimed in claim 3, in which an organic base is used to inhibit 2-alkylation.

5. A process as claimed in claim 4, in which the organic base is used in an amount not exceeding 0.5 mole per mole of starch or hydrolyzed starch used as starting material.

6. A process as claimed in claim 4, 5, in which the organic base used is selected from guanidine, pyridine, cyclohexylamine, ethylenediamine, triethanolamine, monoethanolamine, aniline, phenylenediamine, morpholine, piperidine, pyrrole, piperazine, phenylhydrazine, urea, methylamine, ethylamine, trimethylamine, triethylamine, semicarbazide, pyrrolidine, aminoguanidine and aminophenol.

7. A process as claimed in claim 3, in which the reaction is carried out in the presence of an inorganic salt

8. A process as claimed in claim 7, in which the inorganic salt used is selected from sodium nitrite, sodium hydrogen phosphite, sodium phosphite, aluminium chloride, lithium chloride, titanous chloride, ferrous chloride, manganous chloride, potassium cyanate, potassium chlorate, sodium chloride, sodium chromate, sodium perchlorate, sodium hypophosphite, manganous bromide, sodium nitrate, sodium hypophosphate, sodium carbonate, sodium bicarbonate, sodium pyrosulfite, sodium pyrosulfate, boric acid, metaboric acid, aluminium iodide, sodium borate, aluminium sulfate, potassium aluminium sulfate, sodium thiosulfate, sodium sulfate, magnesium sulfate, potassium phosphate, disodium hydrogenphosphate and sodium phosphate.

9. A process as claimed in any one of claims 3 to 8, in which the starch or hydrolyzed starch used as starting material in the reaction is corn starch, glutinous rice starch, wheat starch, potato starch, waxy maize starch, soluble starch or thin boiling starch.

10. A process as claimed in any one of claims 3 to 9, in which the alkali used as starting material in the reaction is sodium hydroxide, potassium hydroxide, sodium methylate, potassium methylate, sodium ethylate or potassium ethylate.

11. A process for producing a hydroxyethyl starch as claimed in any one of claims 3 to 10, substantially as hereinbefore described and exemplified.

12. A hydroxyethyl starch whenever produced by a process as claimed in any one of claims 3 to 11.

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